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Eye Development: Governed by a Dictator or a Junta?

Minireview

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Recent work in Drosophila has revealed some general principles that guide patterning of imaginal discs, the structures that give rise to the various appendages and the eyes of the adult fly (Lawrence and Struhl, 1996). Although all imaginal discs use the same morphogenetic molecules (hh, wg, and dpp), their relationships have been adjusted to accommodate particular aspects of the morphology of each organ: the flat epithelium of the wing, the long and round leg, or the crystalline array of ommatidia in the eye. Thus, specification of distinct tissue identities occurs at two levels. First, regulatory genes must set up the expression patterns of the signaling molecules and the rules governing their interrelationships. And second, these same, or other, regulatory genes must confer upon the epithelium the ability to respond to these signaling pathways in a specific way to create photoreceptors in the eye or hair cells in the wing. A set of recent observations describing the ability of genes, or combinations of genes, to induce ectopic eyes has opened a new way of thinking about these questions and offers a model of regulation for eye development, not only in Drosophila, but also in vertebrates. Indeed, the same sets of signaling pathways are key regulators during both Drosophila and vertebrate development. It has even been suggested that the relationships between these signaling pathways are conserved for patterning tissues that are not true evolutionary homologs, for instance, the wings of flies and birds (Laufer et al., 1997).

"Master Regulators"

What are the genes that direct development of a tissue toward a specific fate, acting upstream of the signaling pathways described above? Due to the ability of the homeotic selector genes to change one structure into another (e.g., an antenna into a leg), they were classically thought to be responsible for specifying a given structure. However, this seems not to be the case. For instance, comparison of *Hox* gene expression patterns in arthropods with highly different morphologies has shown that these genes merely specify positional values along the axis, and not the type of structure that will develop at a specific location (Akam, 1995). The same selector gene may specify a wing in a species or a halter in another, indicating that the same regionalization function can be interpreted differently to activate the wing or halter programs. One possibility that has recently been offered is that the positional value of *Hox* genes sets in place a small number of regulators that control the entire developmental program for a given organ (Halder et al., 1995).

More recently, a few genes that act as organ regulators, able to determine a specific type of tissue, have been identified. Probably the most striking example is that of Pax6 in the eye. *eyeless* (*ey*), the gene that encodes the Drosophila Pax6 homolog, is essential for eye development, and misexpression of the protein is sufficient to induce well-formed ectopic eyes on almost any of the appendages of the fly (Halder et al., 1995). *ey* was therefore termed "the master regulator of eye development" (Callaerts et al., 1997), i.e., a gene at the top of a hierarchy of developmental genes leading to the correct patterning of the eye disc. The development of ectopic eyes on wings or antennae is highly reminiscent of classical transdetermination experiments where a disc transplanted multiple times from fly to fly can change its fate and become a totally different appendage (Hadorn, 1968). Interestingly, the specific locations in the discs where ectopic *ey* can induce ectopic eyes is very similar to the parts of those discs that can transdetermine to eye. The molecular bases of transdetermination have only recently been investigated, and recent results implicate the interdependence between the *dpp* and *wg* signaling pathways (Johnston and Schubiger, 1996).

Just as a homeotic selector gene is able to specify and impose positional value on a set of cells, a "master regulator" may be able to control a specific organ fate and to impose it on another structure. Instead of directly controlling a whole series of effector genes, the selector genes may control the expression of these "master regulator" genes, which themselves control an entire genetic program. This notion of master regulator implies a high level of specificity that is not found for *ey*: the gene is expressed at multiple locations in the fly and its complete loss of function is lethal. *ey* obviously does more than just control eye development and must act in combination with other genes to determine the eye fate.

Multiple Eye Regulatory Genes: How Many Masters?

If the ability of *ey* to induce ectopic eyes placed it as the master regulator of eye development, recent studies have shown that it is not the only gene able to impose the eye fate upon other tissues (Bonini et al., 1997; Chen et al., 1997 [this issue of *Cell*]; Pignoni et al., 1997 [this issue of *Cell*]). At least five Drosophila genes whose mutations lead to complete loss of the eyes have recently been characterized. These genes are all likely to be regulatory rather than effector genes since *ey* and *eye gone* (*eyg*) encode Pax-like proteins, *sine oculis* (*so*) encodes a divergent homeoprotein, and *eyes absent* (*eya*) and *dachshund* (*dac*) encode novel nuclear proteins. These genes were first believed to act downstream of *ey* since they are required for the formation of ectopic eyes by *ey*.

However, the field is now faced with a quandary since these "downstream" genes can also, alone or in combinations, induce ectopic eyes. *dac* or *eya* alone (Bonini et al., 1997; Chen et al., 1997; Pignoni et al., 1997) can induce small ectopic eyes, though at low penetrance, and mostly in the ventral region of the head or in the antenna. A much more dramatic effect is observed when combinations of two of the genes are used (*da*+*eya* or *so*+*eya*; Chen et al., 1997; Pignoni et al., 1997). In this

case, the effect is fully penetrant and the eyes can form in more locations. This synergy is underlined by the protein–protein interactions that can be observed between the products of *eya* and *so*, and between those of *eya* and *dac* (Chen et al., 1997; Pignoni et al., 1997). This suggests that complexes between these molecules function to pattern the eye, one partner providing targeting through DNA-binding function (so, a homeodomain protein), while the others provide transactivation and/or specificity (dac and eya exhibit activation domains in yeast). However, there must be a more complex set of interactions, as dac and eya would not be expected to act synergistically without a DNA-binding partner. Interestingly, *ey* is required for the ectopic eyes obtained with these genes which are, in fact, able to induce *ey* expression (Chen et al., 1997; Pignoni et al., 1997). This appears to call into question the place of *ey* as a unique regulator on top of a regulatory hierarchy controlling *so*, *eya*, and *dac*. Instead, it suggests a network of interactions among these genes involving reciprocal feedback loops and formation of molecular complexes between their protein products.

Different Requirements of Eye Specification Genes at Multiple Steps of Eye Development

The gain-of-function experiments described above are difficult to interpret, and it is necessary to analyze the genetic epistasis among these genes, and to establish the individual role of each gene during normal eye development. Eye development is a complex process involving several important steps. The first is the specification of the eye imaginal disc during embryonic life. Several genes (*ey*, *so*, and *eyg*) are expressed and likely to be required to define the progenitor regions of the optic lobe and eye disc (Pignoni et al., 1997). However, their epistatic relationship is not known at this stage. The eye disc grows during early larval stages and is later patterned as a wave of photoreceptor differentiation, the morphogenetic furrow (MF), sweeps across the disc. The MF is initiated at the posterior edge of the disc due to the action of dpp and hh, which initiate a circular process of gene induction that allows the reiterative patterning of photoreceptors. The MF progresses anteriorly through the disk due to a dynamic interaction between hh and possibly dpp that is reminiscent of the static interaction observed at the fixed antero-posterior compartment boundary in the wing disc where hh induces *dpp*.

Interestingly, expression of the eye specification genes changes with MF migration: *so*, *eya*, and *dac* are expressed in the posterior region of the eye disc before MF initiation, and are then more highly expressed on both sides of the MF as it moves through the disc. Expression of *ey* overlaps with these genes prior to MF initiation. It is expressed anterior to the moving MF, but its expression quickly disappears posterior to the furrow, as the photoreceptors start their differentiation. In the absence of *ey*, *so*, or *eya*, the disc does not grow properly, the MF does not initiate and its progression is blocked by mutant clones of *so* and *eya*. The disc then degenerates through extensive cell death. Finally, *so* and *eya* are also necessary for photoreceptor differentiation (Pignoni et al., 1997). It has also been suggested that *ey*, which is re-expressed at this stage, plays a role in the terminal differentiation of photoreceptors, directly controlling *rhodopsin* gene expression (Sheng et al., 1997).

During disc morphogenesis, a clear epistatic relationship exists among these genes (Figure 1). *ey* expression does not require *so* or *eya*, while *so* and *eya* do not appear to be expressed in *ey* mutants. *so* and *eya* are both required for each other's expression. Finally, *dac* is downstream of the other genes, as its expression requires their normal function and its absence does not affect their expression (Chen et al., 1997; Pignoni et al., 1997). The loss of *dpp* expression observed in *ey*, *so*, and *eya* mutants does not occur in *dac* mutants: *dac* acts downstream of *dpp* for initiation of the MF, but not for its progression. Thus, these observations, which point toward a linear pathway for turning on the eye specification genes, are not consistent with the gain-of-function experiments that indicate that a regulatory feedback loop exists among these genes.

How to Explain Multiple "Master Regulators"?

Even the most potent eye inducer, *ey*, can only produce eyes on specific parts of the imaginal discs, and each inducer has some propensity to induce eyes in specific subsets of discs. This suggests that only these structures are prone to "transdetermine," while the CNS (where *ey* is normally expressed) or other internal organs cannot. The discs may be the only place where the three pattern signaling pathways (hh, dpp, and wg) intersect and may have retained enough plasticity to allow respecification of their relationship by overexpression of one of the regulators. It must also be noted that most experiments described above used a specific promoter (*dpp* promoter) to drive misexpression in the discs. This pattern of misexpression in the "organizer" region of the imaginal discs juxtaposes expression of *ey* (or other genes) with that of *dpp* and *hh*. As the site of initiation of the MF is defined by the coexpression of dpp and hh, overexpression of an eye specification gene may find there a predisposition to induce an ectopic furrow. Combinations of *so*, *eya*, and *dac* induce eye structures in tissues where *ey* is never expressed, and these ectopic eyes require normal *ey* function. These combinations appear to promote ectopic *ey* expression, at least in the antennal disc, inconsistent with a place of *ey* in a unique upstream position. There are at least three distinct possibilities that can explain how the linear aspect of the pathway can be reconciled with the interactive aspect of the ectopic expression data.

One possibility is that these genes are part of a complex network of genes that constantly cross-regulate. The meaning of this cross-regulation during normal eye development is not clear but may reflect a requirement for a high level of integration between the different functions performed by the various genes to achieve such a complex process as photoreceptor specification. Robust overexpression of a single gene may override previous disc programs and "bootstrap" the entire cascade. Therefore, although the normal epistatic relationship points toward a linear pathway that is required for establishment of the expression pattern, it is clear that a more complex network exists during patterning. This is reminiscent of the myogenic factors that induce muscle development in a broad range of vertebrate cell lines:

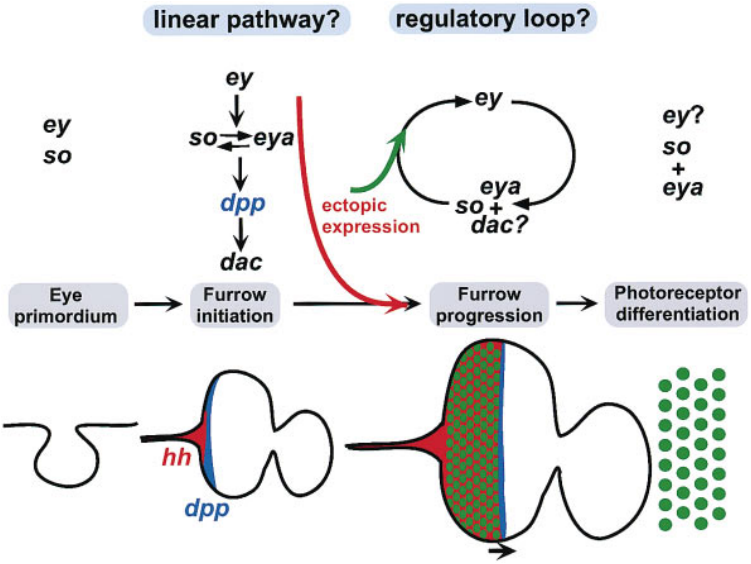

Figure 1. Linear Pathway or Regulatory Loop?

The eye specification genes are required at different stages of eye development, as represented by the gray boxes. Completion of each stage is required for activation of the next developmental program. During embryonic development, *ey* and *so* are expressed in the eye primordium. In the early third instar larval stage, a linear epistatic relationship among all four genes leads to initiation of the morphogenetic furrow (MF). Dpp (blue) at the posterior margin initiates the MF. In late third instar discs, hh (red), expressed in photoreceptors behind the furrow, is responsible for MF migration. A regulatory loop between *ey*, *so*, *eya*, and *dac* may be required for photoreceptor determination. *so* and *eya* (and possibly *ey*) are required for differentiation of the photoreceptors.

Ectopic expression of any gene (or combination of genes) along the linear pathway may activate a downstream developmental stage (red arrow). Or it may enter the regulatory loop during the reiterative morphogenetic process of MF migration (green arrow).

This diagram is meant to illustrate the arguments presented in the text and not to describe only gene interactions or requirements that have been demonstrated experimentally; some of the arrows are speculative.

while these genes can be organized in a linear cascade, they widely cross-regulate and exhibit strong synergy in their function that is supported by protein–protein interactions (Weintraub, 1993).

A second possibility to explain the cross-regulatory interactions between *ey*, *so*, *eya*, and *dac* derives from the idea that most eye regulatory genes are expressed and required multiple times during eye development for specification of the eye disc progenitor, during its patterning and for differentiation of the photoreceptors. Each stage of eye development may involve its own linear pathway, and its completion (i.e., expression of the downstream genes) may lead to activation of the pathway for the next developmental stage. Overexpression of *eya* with *so* or *dac* may bypass an earlier stage in the program where these genes were under the control of *ey*, and be able to activate a later program of *ey* expression in a linear relationship, and not through a real feedback loop (Figure 1). For instance, the whole cascade of genes is required for initiation of furrow movement. Overexpression of downstream genes in the absence of *ey*, but in a region that mimics *hh* and *dpp* expression during furrow initiation (overexpression is achieved with the *dpp* promoter), may start the program for MF migration through *ey* activation. Although this may imply a delay (which is not observed) in the development of the ectopic eyes, it is easy to imagine that, once *ey* has been activated, it is sufficient to quickly start the cascade anew.

A third possibility is that the reiterative nature of eye disc patterning may underline the cross-interactions observed through ectopic expression. Furrow movement requires constant reactivation of the same developmental program that must be linked to earlier events in the preceding row of developing photoreceptors. Thus, the MF may be set in motion by the linear intracellular cascade of genes described above, but its progression may be maintained by a regulatory loop of the same genes that involves communication between subsequent rows of cells. This loop could be interrupted by mutations in any partner, and could be entered at any point by overexpression of a functional complex where the hh and dpp signaling molecules are already present (Figure 1). Interestingly, the pattern of expression of all four eye specification genes changes around the furrow, with increased expression of *so*, *eya*, and *dac* on either side of the MF, and sudden down-regulation of *ey* after the MF: it is possible that, prior to its turning off in differentiated photoreceptors, there is an up-regulation of *ey* expression by *so*, *eya*, and *dac* at the furrow that is essential for eye patterning. The effect of *so*, *eya*, and *dac* on *ey* cannot be detected because the disc is severely affected in these mutant backgrounds. An up-regulation of *ey* and *eya* may be critical for their function since it has been shown that *Pax6* and *eya* mutations in vertebrates are haplo-insufficient (Strachan and Read, 1994; Abdelhak et al., 1997). To understand the significance of *ey* induction by *so*, *eya*, and *dac*, it will be important to distinguish whether these genes induce *ey* expression before any other aspects of eye development, or whether *ey* expression occurs as a later consequence of MF initiation induced by the downstream genes.

An Entire Conserved Cassette for Eye Development?

The fly and vertebrate eyes are obviously very different, and evolutionary studies have suggested that eyes have independently appeared multiple times throughout evolution (Salvini-Plawen and Mayr, 1977). Thus, unique sets of genes might have been expected to direct the

differentiation of these various types of eyes. Still, mutations in the *Pax6* genes of flies, mice, and humans all lead to loss of eyes (Strachan and Read, 1994). To explain why the same gene is controlling very different processes, it has been postulated that the initial role of *Pax6* was to regulate photoreceptor differentiation in a primitive "eye" formed only of photoreceptors (Zuker, 1994; Sheng et al., 1997). As the optical structures of the eye evolved differently in various species, the same regulator was co-opted for each upstream stage of development. In support of this model, *ey* is not only expressed in the embryonic eye primordium and in the developing eye disc, but is re-expressed at the time of photoreceptor terminal differentiation. This terminal phase of *ey* function is likely to represent its ancestral role, which is to control expression of the *rhodopsin* genes through a highly conserved binding site that is found in the *rhodopsin* promoters of most species (Sheng et al., 1997). It is now becoming clear that, in fact, an entire genetic cassette may be used for eye development in many species. Homologs of *so*, *eya*, and *dac* have been described in vertebrates (Chen et al., 1997; Oliver and Gruss, 1997), and they often appear to have overlapping expression patterns in the eye and elsewhere, supporting the model that a molecular complex forms between their gene products. Interestingly, the protein domains that mediate protein–protein contacts between so, eya, and dac are conserved in their vertebrate counterparts. However, as no mutations in genes other than *Pax6* exist in vertebrates, the only functional data so far come from studies in amphibians and fish where ectopic expression of both *Pax6* and *six-3*, a *so* homolog expressed in the eye, can specify ectopic lens fate, but not retinal fate (Altmann et al., 1997; Oliver and Gruss, 1997). Furthermore, although *ey* regulates *so* in Drosophila, six-3 expression is independent from Pax6 in mice, suggesting that the epistasis is different in different species. Confirmation that there is a conserved gene cassette for eye development awaits loss-of-function analyses through the generation of mice mutant for these genes. However, as the expression pattern of all of these genes is much broader than just the eye, it is likely that the specific function of each gene will depend on a combinatorial mechanism.

These results highlight the power of gain-of-function approaches as a way to decipher a complex set of pathways constructed of multiple interactions that classical loss-of-function experiments have problems identifying. Similarly, gain-of-function experiments in Xenopus have been able to induce a new "organizer" in the early embryo and induce axis duplication ("twinning" of the embryo, a transformation that is even more dramatic than eye induction!). Using injection of mRNA encoding signaling molecules or putative transcriptional regulators, these experiments have produced a precise description of interacting signaling pathways (e.g., Wnts and BMPs) and of their role in axis formation during early vertebrate development. In conclusion, multiple genes can activate the eye development pathway, which does not appear to be a simple linear cascade controlled by a master regulator gene. The epistatic relationship may in fact be only required to ensure that the entire cascade of genes is expressed, while their action may involve regulatory loops involving protein–protein contacts. While *ey* still appears to play the most critical role, its function in multiple stages of eye development (or elsewhere), perhaps in all organisms, clearly requires the combinatorial action of other key regulatory genes.

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